UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION
PREVENTION

MEMORANDUM

DATE: April 18, 2011

SUBJECT: Efficacy Review for CaviCide 1;
EPA File Symbol 46781-RE;
DP Barcode: D386574

FROM: Lorilyn M. Montford
Efficacy Evaluation Team
Antimicrobials Division (7510P)

THRU: Tajah Blackburn, Ph.D., Team Leader
Product Science Branch
Antimicrobials Division (7510P)

TO: Marshall Swindell, PM 33/Zebora Johnson
Regulatory Management Branch I
Antimicrobials Division (7510P)

APPLICANT: Metrex Research
1717 West Collins Avenue
Orange, CA 92867

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Didecyldimethylammonium chloride	0.76%
Ethanol	7.50%
Isopropanol	15.00%
Inert Ingredients	76.74%
Total	100.00%

I BACKGROUND

The product, CaviCide 1 (EPA File Symbol 46781-RE), is a new product. The applicant requested to register the product for use as a disinfectant (bactericide, fungicide, tuberculocide, virucide) and deodorizer on hard, non-porous surfaces in commercial, institutional, food preparation, animal care, and hospital or medical environments. Label directions indicate that the product is effective as a disinfectant on pre-cleaned surfaces. Studies were conducted at MICROBIOTEST, located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained EPA Form 8570-4 (Confidential Statement of Formula), twenty seven studies (MRID 483439-11 through 483439-37), Statements of No Data Confidentiality Claims for all twenty seven studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: ambulance equipment, appliance exteriors, barber and salon instruments, basins, bassinets, bathroom fixtures, bathtubs, bed railings, cabinets, cages, carts, chairs, child care equipment, computer keyboards, computers, countertops, cribs, diaper changing stations, diaper pails, diagnostic equipment, doorknobs, examination tables, faucets, filing cabinets, floors, garbage cans, grocery carts, gurneys, hampers, hand rails, handles, headsets, health club equipment, high chairs, infant incubators, infant warmers, kennels, laboratory equipment and surfaces, lamps, light switches, lights, nail care implements, oxygen hoods, patient monitoring equipment, physical therapy equipment, shower stalls, showers, sinks, spine backboards, stethoscopes, stretchers, tables, tanning beds, telephones, toilets, toys, trash cans, trays, ultrasound transducers, urinals, vanity tops, walls, walkers, wheelchairs, and work stations. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: Formica, glass, glazed tile, metal, painted surfaces, plastic (e.g., polycarbonate, polypropylene, polyvinylchloride, polystyrene, vinyl), Plexiglas, and stainless steel. Directions on the proposed label provide the following information regarding use of the product as a disinfectant: Spray product onto pre-cleaned surface, thoroughly wetting area to be disinfected. Allow surface to remain visibly wet for 1 minute (for 3 minutes against Adenovirus type II). Wipe surface using a towel or allow to air dry.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using the AOAC Germicidal Spray Products as Disinfectants Method)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Germicidal Spray Products as Disinfectants Method contains procedures for testing fungicidal activity. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method)

Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as

Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the proposed label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 483439-11 "AOAC Germicidal Spray Test Healthcare," Test Organisms: *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442), for CaviCide 1, by Kathryn D. Dormstetter. Study conducted at MICROBIOTEST. Amended final report date – April 27, 2010. Laboratory Project Identification Number 198-524.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 10-1078, 10-2078, and 10-1011) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. At least one of the product lots tested (i.e., Lot No. 10-1011) was at least 60 days old at the time of testing. The product was received ready-to-use. Cultures of the challenge microorganisms were prepared in accordance. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Sixty (60) glass microscope slide carriers per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 25 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Due to the opacity of the neutralizer with *Pseudomonas aeruginosa*, all *Pseudomonas aeruginosa* subcultures were streaked onto Tryptic Soy Agar and incubated for 24±2 hours at 37±2°C. Following incubation, the subcultures and streaks were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganisms.

2. MRID 483439-12 "AOAC Tuberculocidal Activity of a Germicidal Spray," Test Organism: *Mycobacterium bovis* BCG, for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 31, 2010. Laboratory Project Identification Number 198-529.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation). Two lots (Lot Nos. 10-1078 and 10-2078) of the product, CaviCide 1, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 21-25 day old suspension of the test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to individual tubes of 20 mL of DE Neutralizing Broth. Tubes containing neutralizer were shaken thoroughly after addition of the carrier, as specified in the AOAC method. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 60 days at 37±2°C. The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge organism.

3. MRID 483439-13 "AOAC Tuberculocidal Activity of a Germicidal Spray Confirmatory," Test Organism: *Mycobacterium bovis* BCG, for CaviCide 1, by Angela L. Hollingsworth. Study conducted at MICROBIOTEST. Study completion date – August 31, 2010. Laboratory Project Identification Number 198-534.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika Corporation). Two lots (Lot Nos. 10-1078 and 10-2078) of the product, CaviCide 1, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 21-25 day old suspension of the test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers

were dried for 20 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to individual tubes of 20 mL of DE Neutralizing Broth. Tubes containing neutralizer were shaken thoroughly after addition of the carrier, as specified in the AOAC method. The carriers were transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. All tubes used for secondary transfers were incubated for 60 days at 37±2°C. The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge organism.

4. MRID 483439-14 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Acinetobacter baumannii* (ATCC 19606), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 24, 2010. Laboratory Project Identification Number 198-549.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

5. MRID 483439-15 "AOAC Germicidal Spray Test Supplemental," Test Organism: Methicillin Resistant *Staphylococcus aureus* (ATCC 33592), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 24, 2010. Laboratory Project Identification Number 198-550.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using

the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Following the exposure period, individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See pages 9 and 15 of the laboratory report.

6. MRID 483439-16 "AOAC Germicidal Spray Test Supplemental," Test Organism: Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 24, 2010. Laboratory Project Identification Number 198-551.

This study was conducted against Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for

carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 11 mm) confirmed antibiotic resistance of Vancomycin Resistant *Enterococcus faecalis* (ATCC 51299) to vancomycin. See pages 9 and 15 of the laboratory report.

7. MRID 483439-17 "AOAC Germicidal Spray Test Supplemental," Test Organism: Vancomycin Intermediate *Staphylococcus aureus* (VISA) (ATCC 700699), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 25, 2010. Laboratory Project Identification Number 198-552.

This study was conducted against Vancomycin Intermediate *Staphylococcus aureus* (ATCC 700699). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin Intermediate *Staphylococcus aureus* (ATCC 700699) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 12 mm) confirmed antibiotic resistance of Vancomycin Intermediate *Staphylococcus aureus* (ATCC 700699) to vancomycin. See pages 9 and 15 of the laboratory report.

8. MRID 483439-18 "AOAC Germicidal Spray Test Supplemental," Test Organism: Methicillin Resistant *Staphylococcus epidermidis* (MRSE) (ATCC 51625), for

CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 25, 2010. Laboratory Project Identification Number 198-553.

This study was conducted against Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625) to oxacillin. See pages 9 and 15 of the laboratory report.

9. MRID 483439-19 "AOAC Germicidal Spray Test Fungicidal," Test Organism: *Trichophyton mentagrophytes* (ATCC 9533), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 31, 2010. Laboratory Project Identification Number 198-554.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the suspension was filtered through sterile glass wool to remove the hyphae (which differs from the AOAC method specification to filter the suspension through sterile absorbent cotton). Heat-inactivated horse serum was added to the conidial suspension to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) were inoculated with 0.02 mL of a 10-12 day old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over

the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes of Neopeptone Glucose Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 10 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum counts, carrier counts, sterility, viability, neutralizer effectiveness, fungistasis, and confirmation of the challenge microorganism.

10. MRID 483439-20 "Initial Virucidal Efficacy Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – October 13, 2010. Laboratory Project Identification Number 198-555.

This study, under the direction of Study Director Salimatu Jibril, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing, Inc.), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Initial Virucidal Efficacy Test - Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)," dated July 19, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Following exposure, each plate was neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. Each plate was scraped with a cell scraper to re-suspend the contents, and passed immediately through individual Sephacryl columns. Ten-fold serial dilutions were prepared, using L-15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

11. MRID 483439-21 "Confirmatory Virucidal Efficacy Test Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for CaviCide 1, by Helen Christina. Study conducted at MICROBIOTEST. Study completion date – September 7, 2010. Laboratory Project Identification Number 198-556.

This confirmatory study, under the direction of Study Director Helen Christina, was

conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing, Inc.), using primary duck hepatocytes (ducklings obtained from Metzer Farms) as the host system. One lot (Lot No. 10-2196) of the product, CaviCide 1, was tested according to a MICROBIOTEST protocol titled "Confirmatory Virucidal Efficacy Test - Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)," dated July 19, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained 100% duck serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents, and passed immediately through individual Sephacryl columns. Ten-fold serial dilutions were prepared, using L-15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

12. MRID 483439-22 "Virucidal Efficacy Test Human Immunodeficiency Virus Type 1" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – October 13, 2010. Laboratory Project Identification Number 198-557.

This study was conducted against Human immunodeficiency virus type 1 (obtained from ZeptoMetrix Corporation), using C8166 cells (obtained from the University of Pennsylvania) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Human Immunodeficiency Virus Type 1," dated July 19, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% fetal bovine serum. C8166 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 9-12 days at 36±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL

($TCID_{50}$/mL) was determined using the method of Spearman Karber.

13. MRID 483439-23 "Virucidal Efficacy Test Herpes Simplex Virus Type 1" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 30, 2010. Laboratory Project Identification Number 198-558.

This study was conducted against Herpes simplex virus type 1 (ATCC VR-733), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Herpes Simplex Virus Type 1," dated July 19, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20-21°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 6-8 days at 34±1°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

14. MRID 483439-24 "Virucidal Efficacy Test Herpes Simplex Virus Type 2" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – October 13, 2010. Laboratory Project Identification Number 198-559.

This study was conducted against Herpes simplex virus type 2 (ATCC VR-734), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Herpes Simplex Virus Type 2," dated July 19, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were

inoculated eight-fold with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

15. MRID 483439-25 "Virucidal Efficacy Test Human Influenza A Virus (H3N2)" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 30, 2010. Laboratory Project Identification Number 198-560.

This study was conducted against Human influenza A virus (H3N2) (Strain A/Hong Kong/8/68; obtained from Charles River Laboratories), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Human Influenza A Virus (H3N2)," dated July 22, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following exposure, the plates were neutralized with 2.0 mL of Minimum Essential Medium with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 1.0 µg/mL Trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

16. MRID 483439-26 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Klebsiella pneumoniae* (ATCC 4352), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 25, 2010. Laboratory Project Identification Number 198-561.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to

achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

17. MRID 483439-27 "AOAC Germicidal Spray Test- Supplemental," Test Organism: *Bordetella pertussis* (ATCC BAA-589), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – September 13, 2010. Laboratory Project Identification Number 198-562.

This study was conducted against *Bordetella pertussis* (ATCC BAA-589). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 72±2 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Bordet Gengou Medium Broth with 7% Polysorbate and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. Due to the opacity of the neutralizer, all *Bordetella pertussis* subcultures were streaked onto agar plates and incubated for 72±2 hours and then for another 48±2 hours at 37±2°C (which differs from the AOAC method specification of 48 hours at 37°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

18. MRID 483439-28 "AOAC Germicidal Spray Test Supplemental," Test Organism: *Candida albicans* (ATCC 10231), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 25, 2010. Laboratory Project Identification Number 198-563.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos.

10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the conidial suspension to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes of Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for inoculum counts, carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

19. MRID 483439-29 "AOAC Germicidal Spray Test Supplemental," Test Organism: Extended spectrum β-lactamase *Escherichia coli* (ESBL) (ATCC BAA-196), for CaviCide 1, by M. Hamid Bashir. Study conducted at MICROBIOTEST. Study completion date – August 25, 2010. Laboratory Project Identification Number 198-564.

This study was conducted against Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 30 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of

inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196) to ceftazidime. See pages 9 and 15 of the laboratory report.

20. MRID 483439-30 "Initial Virucidal Efficacy Test, Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 30, 2010. Laboratory Project Identification Number 198-566.

This study, under the direction of Study Director Salimatu Jibril, was conducted against Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories), using MDBK cells (ATCC CCL-22) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Initial Virucidal Efficacy Test - Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C virus)," dated July 22, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate carriers were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, each plate was neutralized with 2.0 mL of horse serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. Each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 5% horse serum. MDBK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

21. MRID 483439-31 "Confirmatory Virucidal Efficacy Test Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)" for CaviCide 1, by Helen Christina. Study conducted at MICROBIOTEST. Study completion date – August 31, 2010. Laboratory Project Identification Number 198-567.

This confirmatory study, under the direction of Study Director Helen Christina, was conducted against Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories), using MDBK cells (ATCC CCL-22) as the host system. One lot (Lot No. 10-1196) of the product, CaviCide 1, was tested according to a MICROBIOTEST protocol titled "Confirmatory Virucidal Efficacy Test - Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)," dated July 22, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate carriers were

sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Following exposure, the plates were neutralized with 2.0 mL of horse serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 5% horse serum. MDBK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

22. MRID 483439-32 "Virucidal Efficacy Test Human Rotavirus" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 30, 2010. Laboratory Project Identification Number 198-568.

This study was conducted against Human rotavirus (ATCC VR-2018), using MA-104 cells (obtained from Charles River Laboratories) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Human Rotavirus," dated July 22, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, the plates were neutralized with 2.0 mL of Minimum Essential Medium with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 1.0 µg/mL Trypsin. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-7 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

23. MRID 483439-33 "Initial Virucidal Efficacy Test Feline Calicivirus (Surrogate for Human Norovirus)" for CaviCide 1, by Helen Christina. Study conducted at MICROBIOTEST. Study completion date – September 7, 2010. Laboratory Project Identification Number 198-571.

This study, under the direction of Study Director Helen Christina, was conducted against Feline calicivirus (ATCC VR-782), using CrFK cells (ATCC CCL-94) as the host system. Two

lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Initial Virucidal Efficacy Test - Feline Calicivirus (Surrogate for Human Norovirus)," dated August 5, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate carriers were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, each plate was neutralized with 2.0 mL of newborn calf serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. Each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. CrFK cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The laboratory reported a failed study set up on August 12, 2010. In that study, the virus culture used contained less than a 5% organic soil load. The laboratory did not accept the assay. These data were not used to evaluate efficacy of the product. Testing was repeated on August 19, 2010. See page 8 of the laboratory report.

24. MRID 483439-34 "Confirmatory Virucidal Efficacy Test Feline Calicivirus (Surrogate for Human Norovirus)" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – October 13, 2010. Laboratory Project Identification Number 198-572.

This confirmatory study, under the direction of Study Director Salimatu Jibril, was conducted against Feline calicivirus (ATCC VR-782), using CrFK cells (ATCC CCL-94) as the host system. One lot (Lot No. 10-2196) of the product, CaviCide 1, was tested according to a MICROBIOTEST protocol titled "Confirmatory Virucidal Efficacy Test - Feline Calicivirus (Surrogate for Human Norovirus)," dated August 5, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. Two replicates were tested. For the single product lot, separate carriers were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 5% newborn calf serum. CrFK cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and

neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

25. MRID 483439-35 "Virucidal Efficacy Test Human Coronavirus (229E strain)" for CaviCide 1, by Salimatu Jibril. Study conducted at MICROBIOTEST. Study completion date – August 30, 2010. Laboratory Project Identification Number 198-575.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using MRC-5 cells (ATCC CCL-171) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Human Coronavirus (229E strain)," dated August 4, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 19°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.004N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in Minimum Essential Medium with 10% fetal bovine serum. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 33±2°C in 5±1% CO_2. The plates were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

26. MRID 483439-36 "Virucidal Efficacy Test Adenovirus Type 2" for CaviCide 1, by Zheng Chen. Study conducted at MICROBIOTEST. Study completion date – November 8, 2010. Laboratory Project Identification Number 198-583.

This study was conducted against Adenovirus type 2 (ATCC VR-846), using A549 cells (ATCC CCL-185) as the host system. Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested according to a MICROBIOTEST protocol titled "Virucidal Efficacy Test - Adenovirus Type 2," dated October 1, 2010 (copy provided). The product was received ready-to-use. The stock virus culture contained at least 5% serum as the organic soil load. Films of virus were prepared by spreading 0.4 mL of virus inoculum over pre-marked bottoms of separate sterile glass Petri dishes. The virus films were dried for 30 minutes at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 6-8 inches from the carrier surface until thoroughly wet. The carriers were allowed to remain wet for 3 minutes at 20°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 1% Polysorbate 80, 0.5% Lecithin, 10% HEPES, and 0.01N HCl. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in

DMEM with 5% fetal bovine serum. A549 cells in multi-well culture dishes were inoculated eight-fold with selected dilutions. The cultures were incubated for 11-14 days at 36±2°C in 5±1% CO_2. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, column titer count, plate recovery count, cytotoxicity, and neutralizer effectiveness/viral interference. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

27. MRID 483439-37 "AOAC Germicidal Spray Test - Supplemental," Test Organism: Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605), for CaviCide 1, by Angela L. Hollingsworth. Study conducted at MICROBIOTEST. Study completion date – November 30, 2010. Laboratory Project Identification Number 198-610.

This study was conducted against Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605). Two lots (Lot Nos. 10-1196 and 10-2196) of the product, CaviCide 1, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready-to-use. A culture of the challenge microorganism was prepared. Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Ten (10) glass microscope slide carriers (1 inch x 3 inch) per product lot were inoculated with 0.02 mL of a 48-54 hour old suspension of test organism. Inoculum was transferred onto a one square inch area of each carrier and immediately spread uniformly over the entire area. The carriers were dried for 34 minutes at 37±2°C. For each lot of product, separate carriers were sprayed with the product (4 pumps) at a distance of 6-8 inches from the carrier surface until uniformly wet. The carriers were allowed to remain wet for 1 minute at 21°C. Following the exposure period, the remaining liquid was drained from each carrier. Individual carriers were transferred to tubes containing Letheen Broth with 7% Polysorbate 80 and 1% Lecithin to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC method. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, two antibiotic disks were added equidistant from one another on the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605) to ceftazidime and gentamicin. See pages 9, 15, and 16 of the laboratory report.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ carrier)
		Lot No. 10-1078	Lot No. 10-2078	Lot No. 10-1011	
1-Minute Exposure Time					
483439-11	*Staphylococcus aureus*	1/60	1/60	0/60	3.6×10^6
	Salmonella enterica	0/60	0/60	0/60	1.4×10^6
	Pseudomonas aeruginosa	0/60	0/60	1/60	5.9×10^6
		Lot No. 10-1196	Lot No. 10-2196		
483439-14	*Acinetobacter baumannii*	0/10	0/10		1.5×10^6
483439-15	Methicillin Resistant *Staphylococcus aureus*	0/10	0/10	---	3.1×10^6
483439-16	Vancomycin Resistant *Enterococcus faecalis*	0/10	0/10	---	4.1×10^6
483439-17	Vancomycin Intermediate *Staphylococcus aureus*	0/10	0/10	---	4.4×10^6
483439-18	Methicillin Resistant *Staphylococcus epidermidis*	0/10	0/10	---	2.0×10^6
483439-19	*Trichophyton mentagrophytes*	0/10	0/10	---	3.6×10^5
483439-26	*Klebsiella pneumoniae*	0/10	0/10	---	2.8×10^6
483439-27	*Bordetella pertussis*	0/10	0/10	---	1.5×10^6
483439-28	*Candida albicans*	0/10	0/10	---	2.9×10^6
483439-29	Extended spectrum β-lactamase *Escherichia coli*	0/10	0/10	---	3.7×10^6
483439-37	~~Multi-Drug Resistant~~ *Acinetobacter baumannii*	0/10	0/10	---	2.0×10^6

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 10-1196	Lot No. 10-2196	

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 10-1196	Lot No. 10-2196	
483439-20	Duck hepatitis B virus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{6.88}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.38 $\log_{10}$	≥ 3.38 $\log_{10}$	
483439-21	Duck hepatitis B virus	10^{-2} to 10^{-3} dilution	---	Cytotoxicity	$10^{6.88}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	---	Complete inactivation	
		$TCID_{50}$/mL	---	$\leq 10^{3.50}$	
		Log reduction	---	≥ 3.38 $\log_{10}$	
483439-22	Human immunodeficiency virus type 1	10^{-2} to 10^{-3} dilution	Cytotoxicity	Cytotoxicity	$10^{8.05}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{4.80}$	$\leq 10^{4.80}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
483439-23	Herpes simplex virus type 1	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{7.68}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 3.88 $\log_{10}$	≥ 3.88 $\log_{10}$	
483439-24	Herpes simplex virus type 2	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.93}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	
		Log reduction	≥ 3.13 $\log_{10}$	≥ 3.13 $\log_{10}$	
483439-25	Human influenza A virus (H3N2)	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{6.50}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.00 $\log_{10}$	≥ 3.00 $\log_{10}$	
483439-30	Bovine viral diarrhea virus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.38}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 10-1196	Lot No. 10-2196	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	
		Log reduction	≥ 3.88 $\log_{10}$	≥ 3.88 $\log_{10}$	
483439-31	Bovine viral diarrhea virus	10^{-2} dilution	Cytotoxicity	---	$10^{6.63}$ $TCID_{50}$/mL
		10^{-3} to 10^{-7} dilutions	Complete inactivation	---	
		$TCID_{50}$/mL	$\leq 10^{2.50}$	---	
		Log reduction	≥ 4.13 $\log_{10}$	---	
483439-32	Human rotavirus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{6.50}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.00 $\log_{10}$	≥ 3.00 $\log_{10}$	
483439-33	Feline calicivirus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{8.05}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{4.80}$	$\leq 10^{4.80}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
483439-34	Feline calicivirus	10^{-2} to 10^{-3} dilutions	---	Cytotoxicity	$10^{7.93}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	---	Complete inactivation	
		$TCID_{50}$/mL	---	$\leq 10^{4.80}$	
		Log reduction	---	≥ 3.13 $\log_{10}$	
483439-35	Human coronavirus	10^{-2} to 10^{-3} dilutions	Cytotoxicity	Cytotoxicity	$10^{6.75}$ $TCID_{50}$/mL
		10^{-4} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.50}$	$\leq 10^{3.50}$	
		Log reduction	≥ 3.25 $\log_{10}$	≥ 3.25 $\log_{10}$	
3-Minute Exposure Time					
483439-36	Adenovirus type 2	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{6.93}$ $TCID_{50}$/mL
		10^{-3} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/mL	$\leq 10^{3.80}$	$\leq 10^{3.80}$	

MRID Number	Organism	Results			Plate Recovery Control
			Lot No. 10-1196	Lot No. 10-2196	
		Log reduction	≥3.13 $\log_{10}$	≥3.13 $\log_{10}$	

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested	
			Lot No. 10-1078, 90 Days	Lot No. 10-2078, 90 Days
1-Minute Exposure Time				
483439-12	*Mycobacterium bovis* BCG Carrier Population: 6.1×10^5 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10
483439-13	*Mycobacterium bovis* BCG Carrier Population: 4.7×10^5 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product, CaviCide 1, as a disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time:

Staphylococcus aureus	MRID 483439-11
Salmonella enterica	MRID 483439-11
Pseudomonas aeruginosa	MRID 483439-11
Acinetobacter baumannii	MRID 483439-14
Methicillin Resistant *Staphylococcus aureus*	MRID 483439-15
Vancomycin Resistant *Enterococcus faecalis*	MRID 483439-16
Vancomycin Intermediate *Staphylococcus aureus*	MRID 483439-17
Methicillin Resistant *Staphylococcus epidermidis*	MRID 483439-18
Klebsiella pneumoniae	MRID 483439-26
Bordetella pertussis	MRID 483439-27
Extended spectrum β-lactamase *Escherichia coli*	MRID 483439-29
Acinetobacter baumannii	MRID 483439-37

Acceptable killing was observed in the subcultures of the required number of carriers tested

against the required number of product lots. In testing against *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. Bacteriostasis controls, when conducted, showed no growth.

2. The submitted efficacy data support the use of the product, CaviCide 1, as a disinfectant with fungicidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time:

Trichophyton mentagrophytes	MRID 483439-19
Candida albicans	MRID 483439-28

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. Fungistasis controls, when conducted, showed no growth.

3. The submitted efficacy data (MRID 483439-12 and -13) support the use of the product, CaviCide 1, as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load for a 1-minute contact time. Complete killing was observed in the subcultures of the required number of carriers against the required number of product lots. No growth was observed in the subcultures of the two extra media. Neutralizer effectiveness testing showed positive growth of the microorganism in all three media. Viability controls were positive for growth. Sterility controls did not show growth.

4. The submitted efficacy data support the use of the product, CaviCide 1, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of at least a 5% organic soil load for the identified contact times:

Duck hepatitis B virus	1 min	MRID 483439-20 and -21
Human immunodeficiency virus type 1	1 min	MRID 483439-22
Herpes simplex virus type 1	1 min	MRID 483439-23
Herpes simplex virus type 2	1 min	MRID 483439-24
Human influenza A virus (H3N2)	1 min	MRID 483439-25
Bovine viral diarrhea virus	1 min	MRID 483439-30 and -31
Human rotavirus	1 min	MRID 483439-32
Feline calicivirus	1 min	MRID 483439-33 and -34
Human coronavirus	1 min	MRID 483439-35
Adenovirus type 2	3 min	MRID 483439-36

Recoverable virus titers of at least 10^4 were achieved. In studies against Duck hepatitis B virus, Feline calicivirus, Human immunodeficiency virus type 1, Human influenza A virus (H3N2), Human coronavirus, and Human rotavirus, cytotoxicity was observed in the 10^{-2} and 10^{-3} dilutions. In studies against Adenovirus type 2, Bovine viral diarrhea virus, Herpes simplex virus type 1, and Herpes simplex virus type 2, cytotoxicity was observed in the 10^{-2} dilutions.

Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against Bovine viral diarrhea virus, Duck hepatitis B virus, and Feline calicivirus, the initial and confirmatory studies were performed at the same laboratory but under the direction of different study directors. Both the initial and confirmatory studies tested two replicates per product lot. The confirmatory study tested one product lot, not the standard two product lots.

VII RECOMMENDATIONS

1. The proposed label claims that the product, CaviCide 1, is an effective disinfectant against the following microorganisms on pre-cleaned, hard, non-porous surfaces with a 1-minute contact time:

Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus
Acinetobacter baumannii
Bordetella pertussis
ESBL *Escherichia coli*
Klebsiella pneumoniae
Methicillin Resistant *Staphylococcus aureus*
Methicillin Resistant *Staphylococcus epidermidis*
~~Multi-drug Resistant~~ *Acinetobacter baumannii*
Vancomycin Intermediate *Staphylococcus aureus*
Vancomycin Resistant *Enterococcus faecalis*

Candida albicans
Trichophyton mentagrophytes

Mycobacterium tuberculosis var: bovis (BCG)

Feline calicivirus (surrogate for Norwalk and Norwalk-like viruses)
Hepatitis B virus
Hepatitis C virus
Herpes simplex virus type 1
Herpes simplex virus type 2
Human coronavirus
Human immunodeficiency virus
Influenza A (H3N2) virus
Norovirus
Rotavirus

These claims are acceptable as they are supported by the submitted data. The Agency is not accepting the claim Multi-Drug Resistant.

2. The proposed label claims that the product, CaviCide 1, is an effective disinfectant against Adenovirus type 2 on pre-cleaned, hard, non-porous surfaces with a 3-minute contact time. This claim is acceptable as it is supported by the submitted data.

3. Methods of application must be limited to spray only, and the label must reflect this pattern of usage. Applications for immersion are unacceptable.

4. The terms "quick", "faster", and "fast" are unacceptable. The Agency has not determined the contact time consistent with these terms.

5. The quantitative claims, Kills 99.9% for bacteria, fungi, and viruses are unacceptable. A 3 $\log_{10}$ reduction is associated with sanitization claims.

6. The claim "faster contact times" is unacceptable. The Agency has not determined the contact time consistent with "faster".

7. Norovirus, not Norwalk virus, is the acceptable term.

8. Adenovirus 2, not Adenovirus II, is the acceptable term.

9. The Agency has not defined household viruses. This claim is unacceptable.

10. The following revisions to the proposed label are recommended:

- On page 3 of the proposed label, change "*Klebsiella pneumonia*" to read "*Klebsiella pneumoniae*."
- On page 9 of the proposed label, change "[Kills viruses in 1 minute]" to read "[Kills viruses in 1 minute (Adenovirus type 2 in 3 minutes)]."
- On page 11 of the proposed label, change "*Bordatella pertussis*" to read "*Bordetella pertussis*."
- On page 11 of the proposed label, add "Multi-drug Resistant *Acinetobacter baumannii*," as it is not listed.
- On page 4 of the proposed label, "aphaeresis" should be changed to read, "apheresis".
- On page 4 of the proposed label, "weight-lifting surfaces" should be changed to read, "hard surface weight lifting surfaces".
- On page 7 of the proposed label, remove the phrase, "on the go".
- On page 8 of the proposed label, remove the phrase, "intermediate level disinfectant".
- On page 11 of the proposed label, the "associated" pathology must be adequately defined in the table listed. The "associated with" pathologies defined as "numerous hospital infections", "hospitals (nosocomial) infections", "difficult-to-treat infections", etc. are unacceptable.